Apolipoprotein A-IV regulates chylomicron metabolism—mechanism and function

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Kohan AB, Wang F, Li X, Bradshaw S, Yang Q, Caldwell JL, Bullock TM, Tso P: Apolipoprotein A-IV regulates chylomicron metabolism—mechanism and function. Am J Physiol Gastrointest Liver Physiol 302: G628–G636, 2012. First published December 29, 2011; doi:10.1152/ajpgi.00225.2011.—Dietary fat is an important mediator of atherosclerosis and obesity. Despite its importance in mediating metabolic disease, there is still much unknown about dietary fat absorption in the intestine and especially the detailed biological roles of intestinal apolipoproteins involved in that process. We were specifically interested in determining the physiological role of the intestinal apolipoprotein A-IV (A-IV) using A-IV knockout (KO) mice. A-IV is stimulated by fat absorption in the intestine and is secreted on nascent chylomicrons into intestinal lymph. We found that A-IV KO mice had reduced plasma triglyceride (TG) and cholesterol levels and that this hypolipidemia persisted on a high-fat diet. A-IV KO did not cause abnormal intestinal lipid absorption, food intake, or adiposity. Additionally, A-IV KO did not cause abnormal liver TG and cholesterol metabolism, as assessed by measuring hepatic lipid content, lipogenic and cholesterol synthetic gene expression, and in vivo VLDL secretion. Instead, A-IV KO resulted in the secretion of larger chylomicrons from the intestine into the lymph, and those chylomicrons were cleared from the plasma more slowly than wild-type chylomicrons. These data suggest that A-IV has a previously unknown role in mediating the metabolism of chylomicrons, and therefore may be important in regulating plasma lipid metabolism.

Despite the importance of dietary fat in mediating metabolic diseases such as atherosclerosis and obesity, there is still much unknown about the physiology of fat absorption in the intestine. Specifically, the detailed biological roles of intestinal apolipoproteins are still emerging, as well as how these proteins are involved in directing the metabolism of dietary fat.

Dietary fat, in the form of triglyceride (TG), travels from the mouth to the stomach where it is metered into the small intestine; once there, the TG is hydrolyzed by pancreatic lipase to form free fatty acids and monoglycerides, which can enter enterocytes of the small intestine. Once inside the enterocyte, the fatty acids are reesterified and assembled along with cholesterol and apolipoproteins into chylomicron particles. Chylomicrons are then secreted from the enterocytes into the intestinal lymph before entering the circulation in the blood.

Chylomicrons are composed of a TG and cholesterol core, coated with apolipoproteins. These apolipoproteins serve three main functions: to emulsify the lipid particles in the aqueous environment of the lymph and blood, to maintain the structural integrity of the chylomicron particle, and finally to direct the metabolism of the particles at the peripheral tissues by binding to cell surface receptors or through enzymatic activities.

Although distinct biological roles have been identified for many apolipoproteins, there is still some uncertainty about the role of apolipoprotein A-IV (A-IV). A-IV is a 46-kDa protein secreted from the small intestine in response to lipid absorption and chylomicron formation (2, 8, 13, 18, 21). Once in the plasma, ~25% of the chylomicron associated A-IV diffuses from the chylomicron and can be found in the lipoprotein-free fraction of the plasma as well as in the HDL pool (13, 14, 30, 37). Therefore, the presence of A-IV in the periphery is uniquely linked to the intestinal absorption and secretion of dietary lipid.

A-IV has been linked with some important anti-atherosclerotic activities, including acting as an antioxidant (32), anti-inflammatory factor (40), and antiatherosclerotic factor (4, 5), as well as a mediator of reverse-cholesterol transport (6, 36) and acute satiety factor (10, 11, 38). Despite in vitro evidence for many of these proposed functions, many of these roles are not unique to A-IV and may not be its primary physiological role.

Lu et al. (24) have suggested that an important role for A-IV is facilitating the packaging of additional lipid into the chylomicron particle, thus promoting the secretion of larger chylomicrons from the intestine. This role is supported by in vitro studies (41), suggesting that A-IV promotes additional lipid to be packaged within each chylomicron particle, since A-IV has hydrophilic amphipathic helices, which stabilize the nascent chylomicron particle. These data are intriguing, especially since the size and composition of chylomicron particles are determinants of their metabolism by peripheral tissues (27, 33). Despite this evidence for a relationship between A-IV and chylomicron formation, the precise details describing the interaction between A-IV and lipid metabolism are not well understood.

To test the role of A-IV, A-IV knockout (KO) mice (generated by Dr. J. L. Breslow; Ref. 43) were backcrossed 15 generations on a C57BL/6 background to avoid an interaction between A-IV KO and a mixed background. The primary phenotype of these A-IV KO mice was a reduction in plasma TG and cholesterol levels, both on chow diet and after an 8-wk HFD challenge. We found that the loss of A-IV did not cause abnormalities in intestinal lipid absorption. The hypolipidemic phenotype is attributable to changes in apolipoprotein C-III (C-III) levels in plasma, which are reduced in the A-IV KO mice, and there are no corresponding changes in hepatic lipid content, lipogenic and cholesterol synthetic gene expression, or...
VLDL secretion. Interestingly, however, A-IV KO significantly delays chylomicron clearance, presumably due to the loss of the A-IV from the chylomicron particle itself. In support of this hypothesis, we found that A-IV KO mice produce chylomicrons that are significantly larger than their WT counterparts. This effect of A-IV on chylomicron formation and metabolism is a novel role for A-IV and supports an intriguing and previously unknown role for A-IV in mediating plasma lipid metabolism.

MATERIALS AND METHODS

Animals. Generation of the A-IV KO mouse was described previously (43) and was kindly provided to us by Dr. J. L. Breslow (Rockefeller University, New York, NY). A-IV mice were backcrossed for >15 generations against the C57BL/6 background. All mice were genotyped by PCR. Adult wild-type C57BL/6 mice were derived from a colony established by mice originally purchased from Jackson Laboratories (Bar Harbor, ME). Mice were housed (3–4 per cage) in a temperature-controlled (21 ± 1°C) vivarium on a 12-h light-dark cycle. All animals received free access to water and chow diet (LM-485 Mouse/Rat Sterilizable Diet; Harlan Laboratories), or for 8 wk, they received either semipurified high-fat diet (HFD) or low-fat diet (LFD) pellets, as described in RESULTS. The HFD contained 19% by weight butter fat and 1% by weight safflower oil (added to prevent essential fatty acid deficiency); the LFD contained 3% by weight butter fat and 1% by weight safflower oil. Male mice were used for all studies age 12–16 wk old. All animal procedures were performed in accordance with the University of Cincinnati Internal Animal Care and Use Committee and in compliance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

Body composition. Fat and lean body mass were determined using an EchoMRI whole body composition analyzer (Echo Medical Systems, Houston, TX). The EchoMRI is a quantitative nuclear magnetic resonance instrument that provides precise measurements of whole body composition, including total body fat, lean mass, and total body water in live mice. Fat and lean mass were calculated as a percentage of total body weight.

Plasma lipids. Plasma lipids were determined after a 5-h fast. To isolate plasma, blood was collected from the tail in heparin-coated microtubes and spun for 5 min. Plasma was used for all enzymatic assays within 24 h of collection and was stored at -80°C until used. Plasma phospholipids and nonesterified fatty acids (NEFA) were analyzed using the phospholipids C and HR Series NEFA HR-2 kits (Wako Chemicals, Richmond, VA). Phospholipids C and HR Series NEFA HR-2 kits (Wako Diagnostics, respectively).

Gene expression. Total RNA was isolated from whole mouse liver after a 5-h fast using Tri-Reagent (Ambion, Austin, TX) according to the manufacturer’s instructions. One-hundred and fifty nanograms of RNA were DNase I-treated (Promega, Madison, WI), and expression of all mRNAs was determined in duplicate by quantitative real-time RT-PCR (Bio-Rad iCycler iQ, Hercules, CA) analysis using Quantitect SYBR green (Qiagen, Valencia, CA) according to the manufacturer’s instructions, using published primer sequences (22, 45). Expression of cyclophilin B was used as a control. The relative amount of mRNA was calculated using the comparative threshold cycle method.

Dietary fat absorption. Dietary fat absorption was measured using the nonabsorbable lipophilic marker sucrose polybenehan, as described previously (15). Before the start of the experimental diet, mice were singly housed in fresh cages, which were changed each day following initiation of the diet. The experimental diet, containing 5% sucrose polybenehan esters and 16% fat was fed ad libitum for 2 days, after which a 10- to 30-mg fecal sample were collected and analyzed by gas chromatography for fatty acid composition (after saponification and methylolation; Ref. 28). The amount of sucrose polybenehan present in the fecal sample was then used to calculate the percent absorption of fat from the diet.

Hepatic lipid analysis. Before the collection of tissue, mice were fasted for 5 h. The liver was carefully dissected, and 100 mg of liver tissue were added to 5 ml of chloroform/methanol (2:1, vol/vol) for Folch extraction (9). After the phases were allowed to separate overnight, 1 ml of the organic phase was removed, dried under nitrogen, and resuspended in ethanol. The total hepatic phospholipid, TG, and cholesterol components were determined by gas chromatography.

Food intake. Food intake was measured over the course of 8 wk, with mice housed four per cage. Diet was weighed before and after administration to each cage. Food intake was calculated as an average intake per animal per day. An experimental number of three (n = 3) was defined as three separate cages of four mice (12 mice total).

Hepatic VLDL secretion. The secretion of hepatic TG was determined as described previously (29). Briefly, mice were weighed and placed in fresh cages, singly housed, with free access to water. Mice were then fasted for 14 h. At time 0, a tail blood sample was collected in heparin-coated microtubes; mice then received an intraperitoneal injection of Poloxamer 407 (1 mg/g body wt in a total volume of 250 μl in PBS). Tail blood was then collected at 1, 2, 3, and 4 h post-Poloxamer injection. Plasma was isolated and assayed for lipids as described above. After 4 h, mice were given fresh cages, water, and diet and allowed to recover.

Isolation of intestinal lymph. Mice were fasted overnight with free access to water. While the animals were under isoflurane anesthesia, the superior mesenteric lymphatic duct was cannulated with polyvinyl chloride tubing, with slight modifications to the procedure described previously (3, 46) and the cannula was secured with a drop of cyanoacrylate glue (Krazy Glue, Columbus, OH). A duodenal cannula was also placed and secured by purse-string suture. After surgery, mice received 5% glucose in saline infusion (0.3 ml/h) via a duodenal cannula to compensate for fluid and electrolyte loss due to lymphatic drainage. Mice recovered for 24 h in Bollman restraint cages at 30°C. All procedures were approved by the University of Cincinnati Internal Animal Care and Use Committee and complied with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

Lipid infusion and lymph collection. To generate doubly labeled chylomicrons, 3 ml of lipid emulsion were prepared, containing 50.1 μCi 3H-labeled glycerol trioleate, 10.2 μCi 14C-cholesterol, and 3 ml of 20% Liposyn II (Hospira, Lake Forest, IL). The morning following the placement of lymphatic and duodenal cannula, mice received an intraduodenal infusion of 0.3 ml of the doubly labeled lipid emulsion. Lymph was then collected on ice for 6 h postinfusion. Each lymph sample was treated with 10% by volume of an antiproteolytic cocktail (0.25 M EDTA, 0.80 mg/ml aprotinin, and 80 U/ml heparin). The lymph collected from A-IV KO mice (n = 4–6) was pooled, as was the lymph from WT mice (n = 3–5), to obtain a large sample for the isolation of chylomicrons.

Isolation of chylomicrons. To isolate the doubly labeled chylomicrons, pooled lymph was subjected to ultracentrifugation using a Beckman 50.3 Ti rotor (Beckman Instruments, Palo Alto, CA) at 25,000 rpm for 40 min at 4°C. The top creamy portion was isolated and the 3H-triglyceride and 14C-cholesterol content was assessed by liquid scintillation counting, and total TG and cholesterol content was assessed by chemical assay (as described above).

Chylomicron clearance assay. Then, 3,000 μg of chylomicron TG (in PBS in a total volume of 100 μl) were injected into the jugular vein of male recipient mice after a 5-h fast. Postinjection, tail blood was taken at 2, 4, 6, 8, 10, and 20 min postinjection. Radioactivity in the plasma was measured by liquid scintillation counting, and the
percent dose was calculated based on a total plasma volume of 4% total body weight.

**Lipid tolerance test.** After a 3-h fast, mice were weighed and received an intraperitoneal injection of Poloxamer 407 (1 mg/g body wt in a total volume of 250 µl in PBS) to block lipoprotein lipase activity. Two hours post-Poloxamer (5 h fast total), mice received an intragastric bolus of lipid (0.1 ml of olive oil). Blood samples were collected from the tail vein before gavage and at 1, 2, 3, and 4 h after gavage. Plasma TG concentration was assayed as described above.

**Determination of chylomicron particle size.** Fresh lymph samples were stained with 2% phosphotungstic acid (pH 6.0) for 5 min, allowed to dry, and then examined with a transmission electron microscope (JEOL, Peabody, MA). Images were documented with an AMT Advantage Plus CD camera, as described previously (23, 37). Eight hundred lipoprotein particles per group were manually measured and counted using printed images of the respective fields of view. Images were evaluated blindly, without the examiner knowing the animals’ genotype, to avoid any bias in the sizing of the lipoprotein particles.

**Western blot analysis.** Fresh lymph samples from both WT and A-IV KO mice were collected and pooled. Lymph TG concentrations were determined as described above. To account for any subtle changes in lymph flow rates, both within the same animal and also between animals of the same group, aliquots of lymph from WT and A-IV KO mice were normalized to TG content, and lymph containing 22 µg of TG (which corresponds to ~10 µl for both groups) was used for Western blotting. Then, 20 µl of 2× SDS sample buffer were added to each sample, and they were boiled for 5 min and then loaded onto a 10% Tris-HCl gel, with a 4% stacking gel (Bio-Rad Laboratories, Hercules, CA). Gels were run at a constant voltage (80 V) until the protein standards were well separated. Proteins were then transferred to a PVDF membrane (Bio-Rad Laboratories, Hercules, CA) for 1 h at a constant current of 350 mA. After nonspecific binding sites on the membranes were blocked for 1 h with a 5% solution of nonfat milk in TBS with 0.1% Tween (TBS-T), membranes were then incubated with either polyclonal goat anti-rat apolipoprotein B antibody (1:5,000 dilution in 5% nonfat milk in TBS-T), polyclonal rabbit anti-mouse apolipoprotein C-III antibody (1:2,000 dilution in 5% nonfat milk in TBS-T), or goat anti-rat apolipoprotein A-I antibody (1:500 dilution in 5% nonfat milk in TBS-T). After incubation with the primary antibody, the blots were washed with nonfat milk in TBS-T and then incubated either with horseradish peroxidase-conjugated goat anti-rabbit antibodies or with horseradish peroxidase-conjugated rabbit anti-goat antibodies (Dako, Denmark) diluted 1:10,000 or 2.5% nonfat milk in TBS-T for 2 h. Detection was achieved by using the enhanced chemiluminescence system (ECL Western Blotting Detection Reagents, Amersham Biosciences, Buckinghamshire, UK), and X-OMAT AR films (Kodak) were used for development and visualization of the membranes.

**Statistics.** Overall statistical significance was determined by two-tailed, unpaired t-tests; differences were considered significant at \( P < 0.05 \). All data are presented as means ± ± ± ± SE. Statistics were performed using GraphPad Prism (version 4.0).

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![Fig. 1. Total plasma triglycerides (TGs), cholesterol, and phospholipids in apolipoprotein A-IV (A-IV) knockout (KO) and wild-type (WT) mice maintained on chow diet (A; \( n = 7–10 \)) or maintained for 8 wk on high-fat diet (HFD; \( B; n = 6 \)). C: plasma nonesterified fatty acids (NEFA) were also measured in both groups. Values are means ± SE. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) vs. WT controls.](http://ajpgi.physiology.org/)

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RESULTS

Lipid levels are reduced in A-IV KO mice, and this effect persists on HFD. The initial deletion of the A-IV gene revealed that plasma lipid levels were dramatically reduced (43). To further characterize and refine this phenotype, and to avoid any contributions of a mixed background to the A-IV KO phenotype, A-IV KO mice were extensively backcrossed against the C57BL/6 genetic background. As shown in Figure 1A, we found that the loss of A-IV had a significant impact on plasma lipid levels, with decreases of 47% in plasma TG, 17% for cholesterol, and 16% for phospholipids, compared with WT controls. This hypolipidemia persists in the face of a HFD challenge, with decreases of 22% in plasma TG, 44% in cholesterol, and 28% in phospholipids, compared with WT controls (Fig. 1B). There were no differences between A-IV KO and WT mice in plasma NEFA on either chow or HFD (Fig. 1C). Therefore, the hypolipidemic phenotype of the A-IV KO mice persists on an almost pure C57BL/6 background and during a HFD challenge.

A-IV KO does not influence food intake or adiposity. The simplest explanation for this hypolipidemic phenotype is that A-IV KO mice eat less or have decreased body weights. As shown in Fig. 2, however, there was actually a slight increase in the body weights of A-IV KO mice vs. WT controls (29.8 vs. 26.4 g, respectively). This did not correspond with changes in either body composition or food intake. These data suggest that A-IV KO does not cause chronic effects on food intake and that the subtle change in body weight is not related to the decrease in plasma lipids.

Dietary fat absorption in A-IV KO mice is not changed. It has been suggested that a major function of A-IV may be in regulating the output of dietary fat from the intestine in chylomicrons (12, 14, 24, 34). To assess the possibility that the loss of A-IV alters the ability of the KO mice to absorb dietary fat, thus leading to lower plasma lipid levels, we used two methods to assess dietary fat absorption. As shown in Fig. 3A, we used a sensitive in vivo detection method in which the fecal excretion of dietary fat is normalized to the excretion of succrose polybehenate, a nonabsorbable lipid marker. There was no difference in fat absorption between A-IV KO and WT mice, with both groups absorbing >95% of the fat in the diet. Next, dietary fat absorption was assessed during a lipid tolerance test. Since mice were treated with Poloxamer 407 before the intragastric lipid load, chylomicrons containing the gavaged lipid cannot be cleared by lipolysis and thus accumulate in the plasma. As shown in Fig. 3B, plasma TG levels rapidly increase over the 3-h study period at the same rate in both A-IV KO and WT mice. Therefore, A-IV KO mice have no defect in dietary fat absorption nor do they have altered output of that TG from the intestine into plasma.

Plasma lipid levels in A-IV KO mice are not influenced by a decrease in hepatic lipid metabolism. Since the liver is a significant contributor to fasting plasma lipid levels, we next assessed whether hepatic lipid metabolism was altered in A-IV KO mice. Hepatic lipid storage was assessed by extracting total hepatic neutral lipids and determining their composition. As shown in Fig. 4A, there was no difference between WT and A-IV KO mice in the amount of total hepatic lipid, regardless of whether those mice were maintained on a either a LFD or HFD. We also measured the composition of the hepatic lipid (Fig. 4B) and found that there was small but statistically significant increase in TG content, coupled with a small but statistically significant decrease in phospholipid content in A-IV KO mice on the LFD; however, there were no differences from WT in the lipid profiles of A-IV KO mice on HFD. Since the lipid profile differences did not persist on the HFD, even though the hypolipidemic phenotype persisted, we concluded that these small changes in the lipid profile of LFD-fed A-IV KO mice might not be physiologically relevant to the lowered blood lipid phenotype. There was no change in cholesterol storage in A-IV KO mice on either diet. These data suggest that the hypolipidemic phenotype of the A-IV KO mice is not due to an increased storage of hepatic lipid.

In addition to serving as a storage site for lipids, the liver is also a main site for TG and cholesterol synthesis. A decrease in liver lipogenic and cholesterol synthetic gene expression could lead to the decreased synthesis and secretion of hepatic VLDL, thus contributing to the hypolipidemic phenotype of the A-IV KO mice. However, we found that there was no difference in the expression of any lipogenic or cholesterol synthetic genes between the A-IV KO and WT mice (data not shown). These data suggest that there is not a defect in the ability of A-IV KO
mouse to synthesize or secrete TG and cholesterol in VLDL from the liver into the blood.

VLDL secretion studies were performed to corroborate these gene expression data and to determine whether there were any differences in in vivo VLDL secretion rates. For these studies, fasted mice were treated with Poloxamer 407, an inhibitor of lipoprotein lipase, which prevents the clearance of VLDL particles secreted from the liver. VLDL secretion can then be assessed by measuring the rise in plasma TG and cholesterol levels over time, since the liver continues to secrete TG and cholesterol-rich VLDL particles that cannot be cleared. As shown in Fig. 5A, A-IV KO mice display the same rate and amount of VLDL TG secretion as WT mice, reaching plasma TG levels of 1,500 mg/dl by the end of the study period. As shown in Fig. 5B, A-IV KO mice and WT mice share the same pattern of cholesterol secretion, reaching a maximum of 202 mg/dl by the end of the study period. Therefore, this in vivo study indicates that A-IV KO does not have an effect on hepatic lipid VLDL secretion. Together with the hepatic lipid and gene expression data, these studies suggest that the hypolipidemic phenotype of the A-IV KO mice is not hepatic in origin.

Chylomicron characterization in A-IV KO mice. The previous study by Weinstock et al. (43) of mixed background A-IV KO mice concluded that the hypolipidemic effect of the A-IV KO coincided with a decrease in C-III expression, since C-III is in a gene cluster with A-IV (19, 20). However, the data supporting this conclusion were not definitive; they overexpressed C-III in A-IV KO mice to above normal levels, which stabilized blood lipid levels. Since it is known that increasing C-III expression will increase plasma lipids (26), it is unclear whether this “rescue” of the A-IV KO phenotype is meaningful. As shown in Fig. 6, we measured C-III levels in our A-IV KO mice and confirmed that C-III levels were reduced 40–60% in lymph (C-III levels were also decreased by the same amount in plasma, data not shown). Additionally, we found that although A-IV KO mice transport the same amount of TG into lymph (Fig. 3), the amount of apolipoprotein B-48 (B-48) and apolipoprotein A-I (A-I) secreted is significantly reduced. The decrease in C-III levels, which persists in our A-IV KO mice, may therefore cause the hypolipidemic phenotype. However, the change in B-48 expression suggests an additional phenotype, in which the chylomicrons secreted by the A-IV KO mouse intestine may be larger and may be metabolized...
differently from WT chylomicrons. This hypothesis is supported by previous studies (24) in intestinal epithelial cell culture, which strongly suggest that A-IV may be necessary for the formation and secretion of chylomicrons.

Chylomicron metabolism in A-IV KO mice is delayed. We next assessed the metabolism of chylomicrons in A-IV KO mice. For these studies, mice received a bolus of radiolabeled chylomicrons through the jugular vein. Postinjection, blood was sampled from the tail vein to assess the clearance of those radiolabeled chylomicrons from the blood. As shown in Fig. 7A, when A-IV KO mice were given chylomicrons isolated from WT mice (WT donor chylomicrons), these chylomicrons were rapidly cleared (with >85% of the chylomicron TG removed from the plasma within the first 2 min postinjection or only 11% remaining). However, when A-IV KO mice were given chylomicrons from A-IV KO mice (A-IV KO donor chylomicrons), these chylomicrons were cleared at a significantly delayed rate compared with the WT donor chylomicrons (with <56% of the chylomicron TG removed from the plasma within the first 2 min postinjection, or 44% remaining). As shown in Fig. 7B, when this experiment was performed in WT mice, the A-IV KO donor chylomicrons were also cleared at a significantly delayed rate, with 63% of the A-IV KO donor chylomicrons remaining at 2 min, compared with only 15% of WT donor chylomicrons. This in vivo study strongly suggests that A-IV may be having a previously unknown effect on chylomicron metabolism.

**Chylomicrons are larger in A-IV KO mice.** We speculated that the delayed chylomicron metabolism might be attributed to a difference in chylomicron size. To evaluate the size of all lipoprotein particles secreted from the intestine into lymph by both A-IV KO and WT mice, we used negative staining and transmission electron microscopy. We found that in WT mice, ~69% of the lipoprotein particles are 50–100 nm in size (the expected size range of VLDL and chylomicrons in mice; Ref. 23), whereas in A-IV KO mice only 31% of their total lymph lipoproteins in this range (Fig. 8). Conversely, only 31% of the lipoproteins counted in WT mice were >100 nm in size, whereas the majority (69%) of the A-IV KO particles ranged from 100–1,000 nm. Thus there is a clear shift towards larger sized chylomicrons in A-IV KO mice during active fat absorption in contrast to WT mice.

**DISCUSSION**

A-IV is a major lipoprotein product of the intestine, making up ~21% of the apolipoprotein content secreted from the intestine (44). Since previous studies have not clearly defined the function of A-IV (using either cell-culture systems or A-IV KO mice of a mixed background), we sought to breed A-IV KO mice onto a C57BL/6 background and to investigate further its physiological role.

In an earlier study by Weinstock et al. (43), in which A-IV KO mice were bred onto a mixed background of strains C57BL/6 and 129, the most striking phenotype of these animals was a decrease in blood TG and cholesterol levels. Whether there was an interaction between A-IV and the 129 background was unclear. Additionally, in light of data from intestinal epithelial cell culture and everted gut sacs suggesting that A-IV may be necessary for the formation of chylomicrons and the secretion of dietary fat from the intestine (24, 25, 34, 41), we hypothesized that the A-IV KO mice may have an additional phenotype that had not yet been described.

After extensively backcrossing our A-IV KO mice onto a C57BL/6 background, we also saw the decreased plasma TG and cholesterol levels; this observation supports the data from the original A-IV KO characterization (43) (Fig. 1). Analysis of these animals after being maintained for 8 wk on a HFD revealed that the hypolipidemia persists. Thus we now know that the genetic background does not make a difference to the plasma phenotype and that the phenotype persists in the face of a dietary challenge.
A-IV has been implicated in the intestinal packaging and secretion of chylomicrons (24), and recently, Simon et al. (34) demonstrated in ex vivo everted gut sacs that the loss of A-IV may cause subtle changes in the regional output of lipid from the intestine into lymph. Using in vivo approaches, we next investigated dietary fat absorption in our A-IV KO mice. We found that A-IV KO mice have normal fat absorption when measured by two different in vivo approaches (Fig. 2). Therefore, lipid is absorbed in the intestine normally in A-IV KO mice, and there are no changes in the secretion of that lipid into lymph. This evidence suggests that A-IV does not modulate overall lipid absorption but that the physiological effect of A-IV KO may instead modulate chylomicron composition or the subsequent metabolism of those chylomicrons. Our data support an intriguing role for A-IV in mediating plasma lipid metabolism that is independent of a change in intestinal lipid transport.

A-IV KO mice also displayed normal food intake and adiposity (Fig. 3). Although A-IV is known to function acutely as a satiety factor (39), we have observed that chronic changes (such as in the A-IV KO mouse) do not have an effect upon total average food intake or weight gain over an extended period (unpublished data). Therefore, the metabolic basis for the lowered plasma TG and cholesterol levels is not due to decreases in food intake or storage in adipose depots. Although the study of Weinstock et al. (43) suggested a role for A-IV in VLDL production, we found no change in hepatic TG output by our A-IV KO mice relative to WT mice with the homogeneous C57BL/6 background (Fig. 5). In addition, the levels of hepatic lipogenic and cholesterol synthetic mRNAs in A-IV KO mice were normal. Plasma NEFA levels were also normal in A-IV KO mice (Fig. 1C). Since NEFA levels are a stimulator of VLDL production (35), this also supports our finding that VLDL production is not affected in A-IV KO mice. The major site of expression of A-IV is the intestine, not the liver (7, 8), so the fact that A-IV KO did not affect hepatic TG storage, synthesis, and VLDL secretion is not surprising. This result does not rule out the possibility, however, that A-IV deficiency can change the clearance of VLDL particles once they are secreted.

The study of Weinstock et al. (43) concluded that the hypolipidemic phenotype of A-IV KO mice was due to de-
creased C-III production, since C-III lies in a gene cluster with A-IV and its expression was changed due to the A-IV KO. C-III is known to be a major determinant of plasma lipid levels due to its inhibitory role on lipoprotein lipase activity, thus inhibiting lipolysis of plasma lipoproteins (1, 26). We also found that C-III levels in A-IV KO mice are depressed by 40–60% in both plasma and lymph (Fig. 6), and we agree that this contributes to the hypolipidemic phenotype. Interesting, however, when we examine the metabolism of chylomicrons derived from A-IV KO mice, clearance of those particles is significantly decreased, even though C-III levels are also decreased in those mice (Fig. 7). This strongly suggests that A-IV itself plays an important role in mediating chylomicron metabolism, since its loss from the chylomicron slows its clearance from the plasma independent from a decrease in C-III expression.

Of the intestinal lipoproteins, A-IV is the most responsive to intestinal lipid absorption and chylomicron secretion (17, 42, 47). It is known that A-IV synthesis and secretion are stimulated two- to threefold by active lipid absorption in the small intestine (17). This stimulation of A-IV is intimately tied to chylomicron formation; when chylomicron formation is inhibited, stimulation of A-IV is abolished (14). Further, when short-chain fatty acids (which are transported via the portal vein rather than in chylomicrons) are infused into the lumen of the intestine, A-IV synthesis and secretion are not stimulated (16, 18). Therefore, the presence of A-IV in the periphery is uniquely linked to the intestinal absorption and secretion of dietary lipid.

Previous data (24) obtained in intestinal epithelial cell culture strongly suggest that A-IV may be necessary for the formation and secretion of chylomicrons. We found that the loss of A-IV did have a significant impact on chylomicron size, since A-IV KO chylomicrons were significantly larger than those from WT mice (Fig. 8). Additionally, the A-IV KO mice secrete less B-48 into lymph than WT mice do, even though the amount of TG secreted is the same (Fig. 6). This B-48-to-TG ratio supports the transmission electron microscope data showing that A-IV KO chylomicrons are larger (31). These data, along with our data showing that chylomicrons from A-IV mice have a decreased rate of clearance, agree with previous reports (33) that larger chylomicrons may be cleared more slowly from plasma.

Neither our study nor that of Weinstock et al. (43) can definitively determine whether the hypolipidemic phenotype of the A-IV KO mice is due solely to C-III levels or may also be a consequence of the loss of A-IV. We found that the hypolipidemic phenotype persists on a clean C57BL/6 background and that in addition to this phenotype the loss of A-IV does not cause alterations in hepatic lipid storage, synthesis, or secretion, nor does it cause defects in total intestinal fat absorption. However, we show here for the first time that a significant role for A-IV may be in modulating both the size and clearance of chylomicrons from the periphery. This novel finding suggests a previously unknown and important function for A-IV in mediating plasma lipid metabolism.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: A.B.K. and P.T. conception and design of research; A.B.K., F.W., X.L., S.B., Q.Y., J.L.–C., and T.M.B. performed experiments; A.B.K. analyzed data; A.B.K. interpreted results of experiments; A.B.K. prepared figures; A.B.K. drafted manuscript; A.B.K., S.B., and P.T. edited and revised manuscript; A.B.K. and P.T. approved final version of manuscript.

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